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TITLE: Function of an Androgen Receptor Coactivator Regulated in Prostate Development and Prostate Cancer

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14. ABSTRACT

We hypothesize that ART-27 affects AR-dependent differentiation of prostate epithelial cells by regulating a subset of AR responsive genes important to prostate growth suppression and differentiation. We further hypothesize that alterations in the level of ART-27 modulates AR activity, which, in turn, affects AR-dependent cell growth regulation *in vivo*. Our aims are to identify ART-27-dependent AR-target genes involved in growth suppression and differentiation and to elucidate the mechanism of regulation of ART-27 expression in prostate cancer. Our approach is to ablate ART-27 protein using siRNA technology followed by gene expression array. Our preliminary findings indicate that loss of ART-27 may result in enhanced expression of genes that are often over-expressed in prostate cancer such as PSA, FKBP5, SOR, KRT18, and CDKN3. Loss of ART-27 also shows enhanced expression of at least one positive regulator of tumor growth, CDKN3.

15. SUBJECT TERMS
prostate, ART-27, cell growth, androgen receptor, cancer

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Function of an Androgen Receptor Coactivator Regulated in Prostate Development and Prostate Cancer

Introduction

Androgen Receptor Trapped clone-27 (ART-27) is a newly described transcriptional coactivator of the androgen receptor (AR) N-terminus. With a predicted molecular weight of ~ 18 kDa, ART-27 binds to a region of AR encompassing the receptor activation functions 1a and 1b (AF-1a and AF-1b) and activates AR-dependent transcription in a dose-dependent manner. Endogenous ART-27 interacts with AR in nuclear extracts of LNCaP cells, and velocity gradient sedimentation of nuclear extracts suggests that native ART-27 is part of a multiprotein complex. ART-27 is expressed in a variety of human tissues, including sites of androgen action, such as prostate. In normal adult human prostate, ART-27 is expressed in AR expressing prostate luminal epithelial cells, in contrast to the stroma, where cells express AR but not ART-27.

During prostate development in humans, ART-27 is expressed in differentiated luminal epithelial cells but is not detected in undifferentiated epithelial cells precursors, suggesting a role for ART-27 in AR-mediated growth suppression and differentiation. Consistent with a growth suppressive function, ART-27 expression levels are negligible in human prostate cancer and regulated expression of ART-27 in the androgen sensitive LNCaP prostate cancer cell line inhibits androgen-mediated cellular proliferation. Moreover, a somatic alteration in AR associated with prostate cancer (AR P340L) shows a diminished capacity to enhance ART-27 mediated AR-transcriptional activation. Thus, ART-27 is a novel AR cofactor that interacts with the AR N-terminus, where it plays a role in facilitating receptor-induced transcriptional activation and, interestingly, displays both cell type and developmental regulation in humans suggestive of a role in AR-dependent differentiation.

Based on these findings, we hypothesize that ART-27 affects AR-dependent differentiation of prostate epithelial cells by regulating a subset of AR responsive genes important to prostate growth suppression and differentiation. We further hypothesize that alterations in the level of ART-27 modulates AR activity, which, in turn, affect AR-dependent cell growth regulation *in vivo*.

Our aims are to identify ART-27-dependent AR-target genes involved in growth suppression and differentiation and to elucidate the mechanism of regulation of ART-27 expression in prostate cancer.

Key Research Accomplishments

The original grant outlined two tasks in the statement of work. Each task is listed below followed by a description of the research progress relevant to the task.

Task 1. Identify ART-27-dependent AR- target genes.

a. Evaluate the effect of reduced ART-27 expression on AR target genes in prostate cancer cells.

To evaluate the effect of ART-27 on AR target gene expression in prostate cancer cells conditions for small interfering RNA (siRNA) "knock-down" of the ART-27 protein were

optimized. LNCaP cells were treated with non-specific siRNA or ART-27 siRNA (SMARTpool, Dharmacon, Lafayette, Colorado) in the presence or absence of R1881. RNA was then isolated from the four sets of treated cells, labeled and utilized for gene array as shown in the schmatic below.

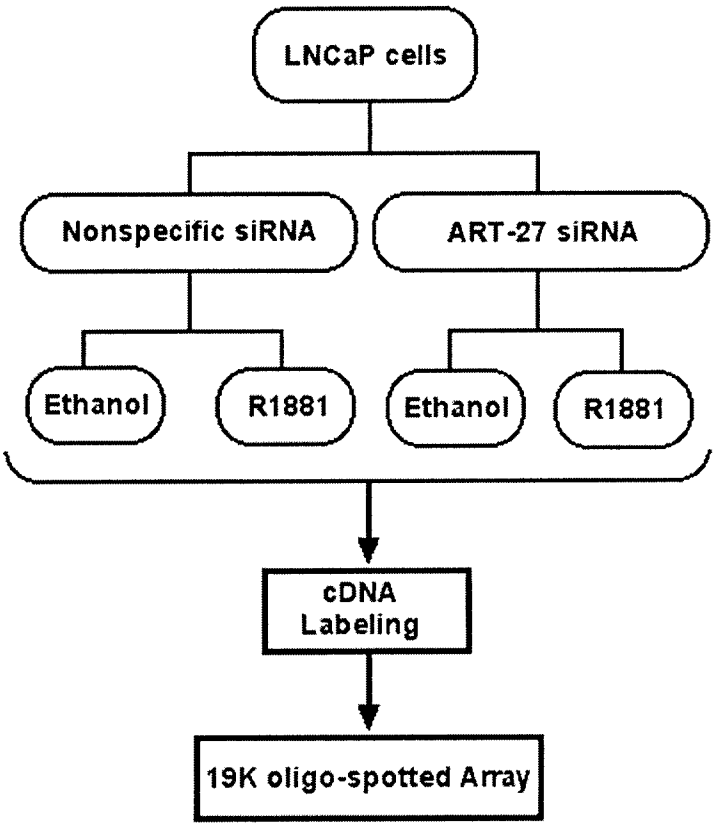

Figure 1. Scheme for ART-27 siRNA "knock-down" and subsequent gene array.

Following hybridization to the gene chips analysis was conducted to identify genes that were androgen and or ART-27 dependent for transcription. Primers were designed against genes of interest and real time PCR was conducted to verify the gene array findings. Figure 2 (Supporting Data) shows a depiction of rtPCR conducted on RNA from the four conditions outlined in Figure 1. The genes shown in Figure 2A (Supporting Data) are androgen dependent and many of these genes appear to be up-regulated in the absence of ART-27. This suggests that ART-27 may act as an androgen dependent repressor for a variety of genes including PSA, Nkx3.1, FKBP5, KRT18, and SEC24D. As markers of prostate growth and differentiation, regulation of PSA and Nkx3.1 is interesting. We are currently verifying these results by doing sequential knock-downs of ART-27 and the repeating the expression array. KRT18 mRNA levels were also increased upon loss of ART-27 in an andorgen-dependent manner. KRT18 is a luminal marker that is highly expressed in prostate cancer (Oncomine). Figure 2B (Supporting Data) shows rtPCR of additional genes, many of which are affected by loss of ART-27. Of note, are CDKN3/KAP that dephosphorylates CDK2 in normal cells and is over-expressed in prostate

and breast cancer cells. In addition, EP400/p400 appears to be up-regulated by loss of ART-27 in an androgen independent manner. EP400 is a SWI/SNF2 family member, a component of the TIP60 HAT complex, and depletion of EP400 results in premature senescence.

In conclusion:

- Loss of ART-27 may result in enhanced expression of genes that are often over-expressed in prostate cancer such as PSA, FKBP5, SOR, KRT18, and CDKN3.

- Loss of ART-27 shows enhanced expression of at least one positive regulator of tumor growth, CDKN3.

b. Perform chromatin immunoprecipitations to assess the presence of ART-27 on promoters identified in Aim 1a.

The grant stated that "I estimate that that Aim 1a (identification of ART-27 dependent AR-target genes) will take 1.5 years to complete at which time we can begin Aim 1b (ChIP on targets identified in a), which will also take approximately 1.5 years." Therefore this aim has not yet begun although if PSA is a target of ART-27, the aim will be relatively straightforward since we have worked out the conditions for ChIP analysis of the PSA promoter in the presence and absence of R1881 using AR antibody in preparation for this aim.

c. Differentiate NRP-152 cells in the presence of ART-27 small interfering RNA (siRNA) duplexes or non-specific siRNA to evaluate the effect of ART-27 on differentiation and target gene transcription. NRP-152 cells are rat prostatic basal epithelial cells that can be differentiated toward a luminal phenotype. Consistent with its *in vivo* expression, ART-27 expression is evident only in the differentiated cells.

We have treated NRP-152 cells with ART-27 siRNA and observed no morphological effect. We are in the process of assessing whether ART-27 remained ablated throughout the course of the 8-day treatment.

Task 2. Examine the regulation of ART-27 expression in prostate cancer.

To determine if ART-27 is transcriptionally down-regulated in prostate cancer we took advantage of a tissue array database created by Dr. William Gerald at Memorial Sloan Kettering Cancer Institute (New York, New York). The results shown in Table 1 (below) show that there is no difference in ART-27 transcript in normal prostate versus prostate cancer samples. This is verified by in situ hybridization done in collaboration with Dr. Peng Lee that also show no difference in ART-27 mRNA between regions of benign (BN) and prostate Cancer (CA), see Figure 3 in Supporting Data.

		Average	
pooled reference	1379		
bulk prostate normal	2109		
prostate stroma normal	1518		
prostate epithelium normal	2248		
normal		1856	n=8
primary recurrent		1992	n=37
primary nonrecurrent		2083	n=41
metastatic prostate cancer		2073	n=5
metastatic prostate cancer androgen independent		2605	n=3
LNCAP with androgen	3028		
LNCAP without androgen	2226		

Table 2. Shows the levels of ART-27 mRNA in prostate cancer relative to normal prostate.

REPORTABLE OUTCOMES

Abstracts and Publications, 2004-2005

Ha, S, Swenson, N, Huang, H-Y, Shapiro, E, Taneja, S Garabedian, MJ, and **Logan,** SK Androgen receptor coactivator ART-27, in androgen-mediated cell growth, cancer and development. Keystone Annual Meeting, Keystone, Colorado, February 28, 2004, abstract

Wenhui Li , Susan K. **Logan** and Michael J. Garabedian. An androgen receptor mutation isolated from a prostate cancer patient displays aberrant ART-27 coactivator function Keystone Annual Meeting, Colorado , 2004, abstract

Taneja, S., Ha, S., Swenson, N., Rome, S., Walden, P., Huang, H.Y., Shapiro, E., Garabedian, M.J., and **Logan**, S.K. Androgen receptor coactivator, ART-27, in androgen-mediated cell growth, cancer and development. J Biol Chem. (2004) 279, 13944.

Li, W., Claudio, Cavasotto, T., Ha, S., Cordoza, Dang, T., Taneja, S., **Logan**, S.K. and Garabedian, M.J. An androgen receptor mutation identified in prostate cancer displays aberrant ART-27 coactivator function. Mol Endocrinol. 2005 May 26; [Epub ahead of print]

Taneja, S.S., Ha, S., Swenson, N.K., Huang, H.Y., Lee, P., Melamed, J., Shapiro, E., Garabedian, M.J. and **Logan**, S.K. Cell specific regulation of androgen receptor phosphorylation *in vivo*. J Biol Chem. 2005 Oct 6; [Epub ahead of print]

Research training during the period of grant support:

Courtney Phillips	Urology resident	7/1/03-3/31/04	resident, NYU Dept. of Urology
David Fenig	Urology resident	7/1/04-3/31/05	resident, NYU Dept. of Urology
Minotti Hiremath	graduate student tutorial, weekly	4/6/05-6/1/05	
Jonathan Leventhal	NYU summer student	5/17-05- 7/17/05	
Marie Sol Flaherty	Sackler rotation student	1/01/05- 4/01/05	
Irene Parker	Sackler student student	4/18/05- 7/31/05	
Jessica Feig	summer student	6/1/05-9/1/05	

SUPPORTING DATA

rt-PCR of ART-27 target genes

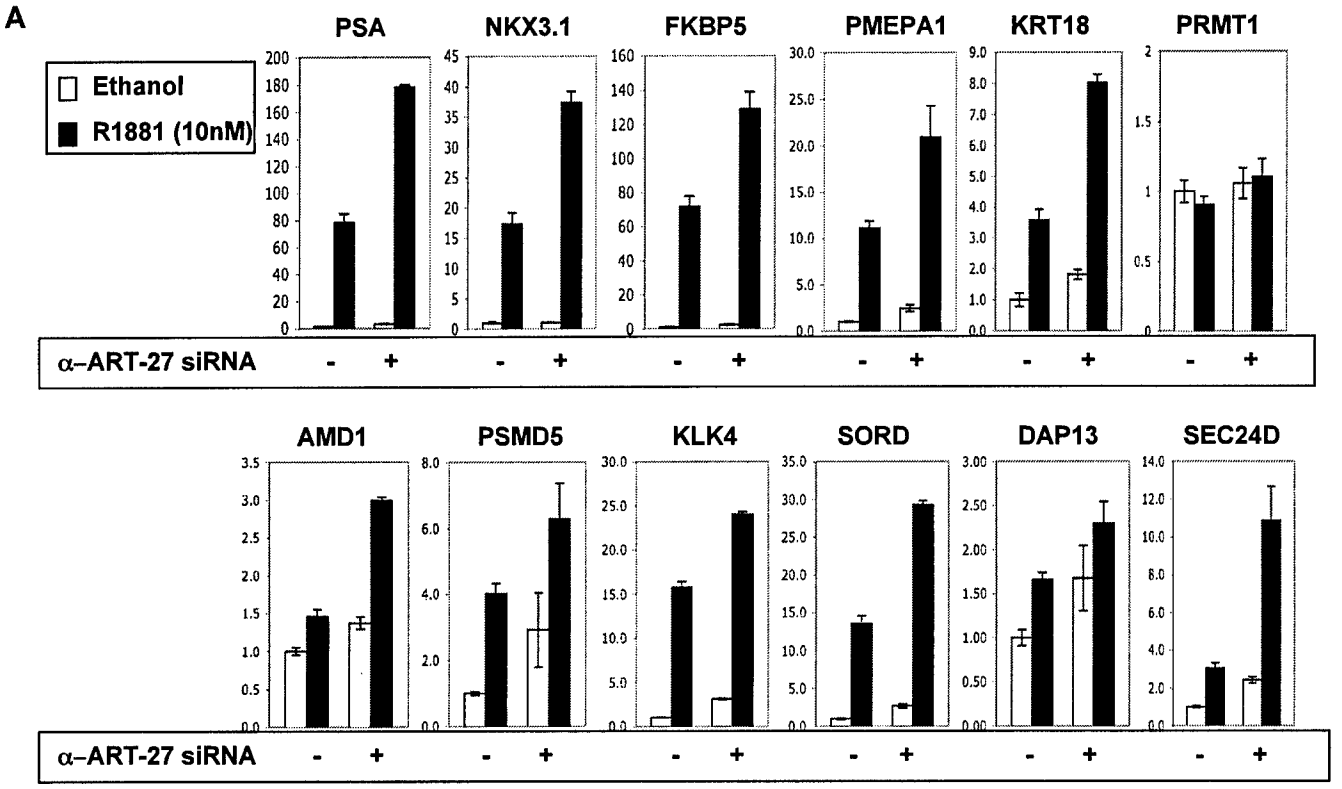

Figure 2A. Shows rt-PCR of putative ART-27 target genes. LNCaP cells were treated with or without ART-27 siRNA in the presence of absence of R1881 as indicated. RNA was made from the cells and used to do gene array. Targets identified by gene array were further investigated by rt-PCR which was quantified and represented here.

rt-PCR of ART-27 target genes

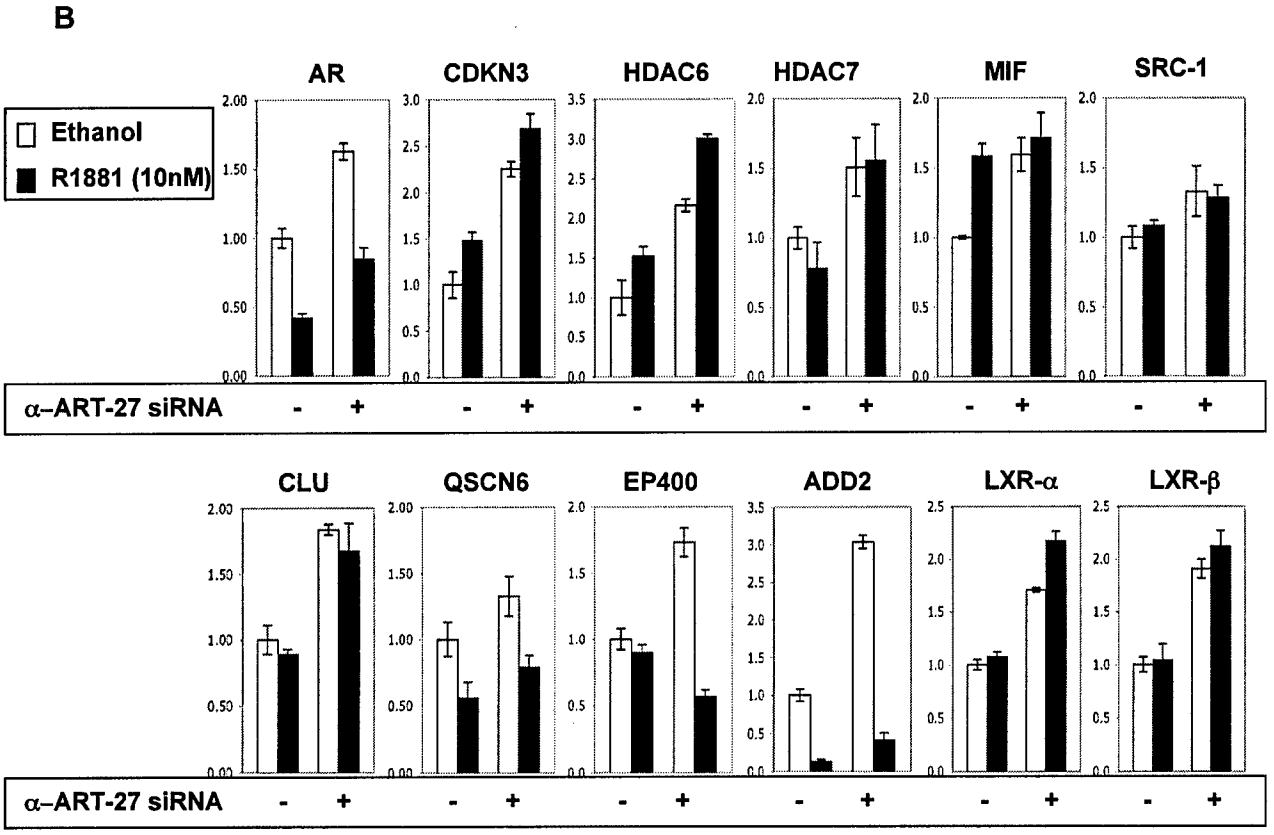

Figure 2B. Shows rt-PCR of putative ART-27 target genes. LNCaP cells were treated with or without ART-27 siRNA in the presence of absence of R1881 as indicated. RNA was made from the cells and used to do gene array. Targets identified by gene array were further investigated by rt-PCR which was quantified and represented here.

ART-27 in situ hybridization in prostate tissue

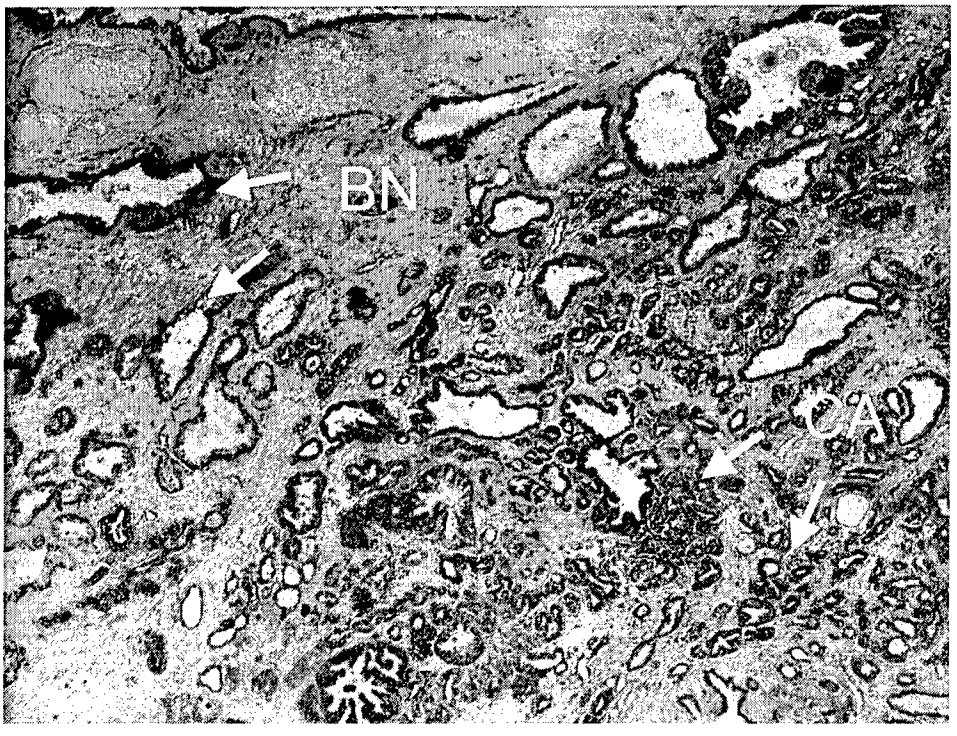

Figure 3. Shows in situ hybridization using a probe that recognizes ART-27 mRNA. Positive reactivity is indicated by the dark purple stain. The large benign glands (BN) show similar levels of ART-27 mRNA as the small cancerous cancerous glands (CA).